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Cancer incidence among pesticide applicators exposed to metolachlor in the Agricultural Health Study

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ABSTRACT

Metolachlor is one of the most widely used herbicides in the United States. We evaluated the incidence of cancer among pesticide applicators exposed to metolachlor in the Agricultural Health Study, a prospective cohort study of licensed pesticide applicators in Iowa and North Carolina. A total of 50,193 pesticide applicators were included. Detailed information on pesticide exposure and lifestyle factors was obtained from self-administered enrollment questionnaires completed between 1993 and 1997; average length of follow-up was 7.33 years. Two metolachlor exposure metrics were used : (*i*) lifetime days personally mixed or applied metolachlor and (*ii*) intensity-weighted lifetime days (lifetime days × an intensity level). Poisson regression analysis was used to estimate relative risks (RR) and 95% confidence intervals (95%CI) for cancer subtypes by tertiles of metolachlor exposure. No clear risk for any cancer subtype was found for exposure to metolachlor. A significantly decreased RR was found for prostate cancer in the highest category of lifetime days exposure (RR = 0.59; 95%CI, 0.39-0.89) and in the second highest category of intensity-weighted lifetime days exposure (RR = 0.66; 95%CI, 0.45-0.97); however, the test for trend was not significant for either exposure metric. A nonsignificantly increased risk was found for lung cancer with lifetime days exposure in the highest

category (RR = 2.37; 95%CI, 0.97-5.82, *p*-trend = 0.03) but not with intensity-weighted lifetime days. Given the widespread use of metolachlor and the frequent detection of metolachlor in both surface and ground water, future analyses of the AHS will allow further examination of long-term health effects, including lung cancer and the less common cancers. © 2006 Wiley-Liss, Inc.

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ARTICLE TEXT

Metolachlor, a chloroacetanilide herbicide first registered in the United States in 1976, is used for general weed control in many agricultural food and feed crops (mainly corn, soybeans and sorghum), turf and other residential applications. It is one of the most widely used herbicides in the United States, and in 1991 the EPA (IRIS system) classified metolachlor (CAS Number 51218-45-2) as a Group C "possible human carcinogen." This was based on a significant increase in liver neoplastic nodules observed at the highest dose level of 3,000 ppm for 104 weeks among female, but not male, rats.[1] No carcinogenic effects were observed in 2 chronic studies in Charles River CD-1 mice.[1] It is hypothesized that the carcinogenicity of metolachlor involves a complex metabolic activation pathway leading to a DNA-reactive dialkylbenzoquinone imine.[2]

To date, only 2 epidemiologic studies have provided information on the carcinogenicity of metolachlor in humans. Alavanja *et al.*[3][4] investigated lung cancer risk[3] and prostate cancer risk[4] with respect to exposure to each of the 50 pesticides measured in the Agricultural Health Study (AHS), one of which was metolachlor. An increased risk for lung cancer occurred among applicators reporting the use of metolachlor. No association was found for prostate cancer. In another study from the AHS cohort, Lee *et al.*[5] found an increased risk for hematopoetic cancers with lifetime days exposure to another chloroacetanilide herbicide, alachlor.

Despite the potential for exposure to applicators during metolachlor use, possible exposure to the general population from widespread use, and frequent detection of metolachlor in surface and groundwater,[6] there is limited information concerning its potential mutagenicity or carcinogenicity. Most of this information is based on mammalian and nonmammalian bioassays.[7][8][9][10][11][12][13][14][15] We investigated site specific cancer incidence and risk among pesticide applicators exposed to metolachlor in the Agricultural Health Study cohort to provide additional information on this important agricultural chemical. This study extends previous analyses in this cohort for lung and prostate cancers and examines the relationship with metolachlor for additional cancer sites.

Methods

Cohort enrollment and follow-up

The AHS is a prospective cohort study, composed of 57,311 private and commercial applicators who are licensed to apply restricted use pesticides and living in Iowa or North Carolina (82.4% of eligible applicators in both states enrolled). This cohort was established to evaluate findings from previous studies that suggested that farmers may be at elevated risk for a few cancers.[16] Recruitment of the cohort occurred between 1993 and 1997.[17] Cohort members were matched to cancer registry files in Iowa and North Carolina for case identification and to the state death registries and the National Death Index to ascertain vital status. For this study, incident cancers were identified for the time period from the date of enrollment to December 31, 2002 and were coded according to the International Classification of Diseases for Oncology (ICD-O-2). Cohort members alive, but no longer residing in Iowa or North Carolina (n = 1,080), were identified through current address records of the Internal Revenue Service (address information only), Motor Vehicle Registration offices and pesticide license registries of the state agricultural departments and were censored in the year that they left the state. Individuals were followed until the earliest of (*i*) first primary cancer diagnosis of any type, (*ii*) death, (*iii*) date they left the state, or December 31, 2002. The average time of follow-up is 7.33 years; 94% of the cohort has been followed up for at least 5 years. All participants provided verbal informed consent, and institutional review boards of the National Cancer Institute, Battelle (the field station in North Carolina), the University of Iowa (the field station in Iowa) and Westat (coordinating center for the study) approved the protocol.

Exposure assessment

A self-administered enrollment questionnaire collected comprehensive exposure data on 22 pesticides,

ever/never use information for 28 more pesticides, information on use of personal protective equipment (PPE), pesticide application methods, pesticide mixing, equipment repair, smoking, alcohol consumption, cancer history of first degree relatives and basic demographic data.[18] Applicators who completed this questionnaire were also given a self-administered take-home questionnaire, which sought additional information on occupational exposures. Reliability of pesticide reporting has been evaluated, which was in the 70-90% range for ever/never use of individual pesticides and in the 50-60% range for duration, frequency and decade of use.[19] The questionnaires may be accessed at http://www.aghealth.org.

Questionnaire data from enrollment and measurement data from the published pesticide exposure literature were used to calculate estimated intensity of exposure to individual pesticides using the following algorithm: Intensity level = {[mixing status + application method + equipment repair status] × personal protective equipment (PPE) use}.[20] The scores assigned to each factor in the intensity level algorithm were not assigned as nominal or ordinal values, but were weighted to reflect the intensity of exposure as described in the literature. Mixing status (mix) was a 3 level variable, based on never mixing, personally mixing less than 50% of the time, and personally mixing more than 50% of the time (mix = 0, 3, 9, respectively). Application method (applic) was a 6 level variable, which, for herbicides, was based on never applying, use of aerial-aircraft or distribution of tablets, application in furrow, use of boom on tractor, use of backpack and use of hand spray (applic = 0, 1, 2, 3, 8, 9, respectively). Equipment repair status (repair) was a two level variable, based on not repairing or repairing (repair = 0, 2 respectively). PPE was an 8 level variable based on type and amount of PPE generally worn.[20]

We constructed 2 lifetime metolachlor exposure metrics, each categorized into tertiles, for this analysis: The first metric was lifetime exposure-days (based on the number of years personally applied or mixed metolachlor multiplied by the number days in an average year an applicator personally mixed or applied metolachlor). We used the midpoints of the questionnaire categories to calculate the product of years of use × days per year (tertiles: ≤20, 21-56, >56). The second metric was intensity-weighted lifetime exposure-days, which was the product of lifetime exposure-days and intensity level (i.e., years of use × days per year × intensity level; tertiles: ≤103, >103-362, >362). For the more common cancers we split the top tertile at the median.

Data analysis

Individuals with prevalent cancers, identified at or prior to the time of enrollment (n = 1,075), and applicators who did not provide information on metolachlor use (n = 6,043) were excluded from this analysis, leaving 50,193 applicators. Analyses of first primary incident cancer cases enabled us to obtain exposure data from each case prior to the onset of cancer.

We used Poisson regression analyses for individual cancer sites to estimate rate ratios (RR) associated with tertiles of lifetime exposure days or intensity-weighted exposure days. We investigated all cancer sites classified under ICD-O-2, but in the table we present only cancers for which there were at least 20 exposed cases and 5 cases in the referent category, after accounting for missing covariate data. Rate ratios were adjusted for age at enrollment (quartiles), sex, race (white, nonwhite), alcohol consumption in the last year (ever/never), cigarette smoking (never/low/high: the median value of pack-years among smokers was used to classify low and high categories of smokers), family history of cancer in first degree relatives (yes/no), applicator status (private, commercial) and state of residence (Iowa/North Carolina). Because of potential concomitant exposure to other pesticides, we adjusted RRs for exposure to those pesticides whose use was most highly correlated with metolachlor (i.e., $r \geq 0.50$): cyanazine (r = 0.56), *s*-ethyl dipropylthiocarbamate (EPTC) (r = 0.54), alachlor (r = 0.53), imazethapyr (r = 0.52) and trifluralin (r = 0.50). The exposure levels of these 5 pesticides were categorized as never, low, and high; for each pesticide, the cut point between low and high exposure was set at the median of intensity-weighted exposure-days. We analyzed exposure-response trends by including the midpoint of each tertile as a continuous variable in the model and testing for the statistical significance of the slope.

To ensure the use of the more appropriate reference group - either applicators who never applied metolachlor (hereafter referred to as "non-metolachlor exposed applicators") or applicators in the lowest exposure tertile of metolachlor (hereafter referred to as "low-metolachlor exposed applicators") - we carried out a comparison of baseline characteristics between different types of pesticide applicators: (*i*) non-metolachlor exposed applicators, (*ii*) low-metolachlor exposed applicators and (*iii*) exposed applicators with metolachlor exposure in the highest 2 tertiles of lifetime exposure-days. Applicators with baseline characteristics more similar to those of the applicators in the higher exposure group would be more appropriate as a reference group for the Poisson regression analyses. Difference with respect to baseline characteristics might introduce residual confounding from a variety of unidentified sources. We determined that the low-metolachlor exposed applicators were somewhat more similar to the applicators in the 2 highest tertiles than the non-metolachlor exposed applicators. However, using the low exposed category as the referent also has limitations, so we have opted to

perform analyses using both the low exposed tertile and the non-exposed as referents.

All statistical analyses were conducted in the Stata program (version 8.0).[21] We used the P1REL0502 release of the AHS database.

Results

Selected characteristics of the metolachlor-exposed and non-metolachlor exposed applicators are presented in Table I. Among 50,193 subjects with complete exposure information, 23,395 (47%) reported ever having personally applied or mixed metolachlor and had complete data on lifetime days of exposure; these people contributed a total of 169,158 person-years to the analysis. The cohort, both exposed and nonexposed, was comprised of primarily white, male private applicators. This is a population with relatively low smoking rates; i.e., in both the exposed and nonexposed groups, about half of the subjects reported that they had never smoked. There was little difference between metolachlor exposed and non-metolachlor exposed subjects with respect to age, family history of cancer in a first degree relative, smoking, having ever lived on a farm or production of sorghum. However, about 80% of metolachlor-exposed applicators were from Iowa and 20% from North Carolina, while the distribution among non-metolachlor exposed applicators was about 60% from Iowa, 40% from North Carolina. At the time of interview about 85% of metolachlor-exposed applicators were involved in production of corn and 80% in soybean production, while the percentages were 60 and 55, respectively for applicators not exposed to metolachlor. There were more similarities with respect to alcohol consumption and education for the metolachlor exposed groups (lowest tertile and higher 2 tertiles) than for the non-exposed group and the higher exposed group.

Table I. Selected Characteristics of Applicators by Metolachlor Exposure in The Agricultural Health Study (Based on 1993-1997 Enrollment Data)

	Nonexposed group (*n* = 27,918)		Lowest exposed group[1] (*n* = 7,202)		Higher exposed group[2] (*n* = 15,991)	
Characteristics	**Number**	**%**	**Number**	**%**	**Number**	**%**
Age (years)						
<40	9,123	32.7	2,167	30.1	5,674	35.5
40-49	7,215	26.1	2,180	30.3	4,953	31.0
50-59	5,714	20.5	1,551	21.5	3,123	19.5
≥60	5,809	20.8	1,304	18.1	2,241	14.0
Gender						
Male	26,803	96.0	7,121	98.9	15,879	99.3
Female	1,115	4.0	81	1.1	112	0.7
Family history of cancer						
No	15,756	56.4	3,968	54.3	8,906	55.7
Yes	10,204	36.6	2,935	40.8	6,334	39.6
Missing	1,958	7.0	359	5.0	751	4.7
Smoking history						
Never	14,398	51.6	4,020	55.2	8,833	55.2
Low (<11.25 pack-years)	6,015	21.6	1,516	22.4	3,584	22.4
High (≥11.25 pack-years)	6,380	22.9	1,469	20.1	3,208	20.1
Missing	1,125	4.0	197	2.7	366	2.3
Alcohol consumption						
Never in past year	9,791	35.1	2,035	28.5	3,915	24.5
Ever in past year	17,609	63.1	5,098	70.8	11,942	74.7
Missing	518	1.9	69	1.0	134	0.8
Education						

High school/GED	15,827	56.7	3,855	53.5	8,170	51.1
>High school	11,438	41.0	3,233	44.9	7,535	47.1
Missing	653	2.3	114	1.6	280	1.8
Race						
White	27,118	97.5	7,060	98.2	15,698	98.3
Non-white	709	2.6	131	1.8	266	1.7
Missing	91	0.3	11	0.2	27	0.2
Currently own/live on a farm						
No	3,496	12.5	214	3.0	1,214	7.6
Yes	23,928	85.7	6,949	96.5	14,701	91.9
Missing	494	1.8	39	0.5	76	0.5
State of residence						
Iowa	16,636	59.6	5,580	77.5	12,518	78.3
North Carolina	11,282	40.4	1,622	22.5	3,473	21.7
Applicator type						
Private	25,321	90.7	6,948	96.5	14,169	88.6
Commercial	2,597	9.3	254	3.5	1,822	11.4
Corn production						
No	11,299	40.5	1,098	15.3	2,777	17.4
Yes	16,619	59.5	6,104	84.8	13,214	82.6
Sorghum production						
No	27,513	98.6	7,087	98.4	15,689	98.1
Yes	405	1.5	115	1.6	302	1.9
Soybean production						
No	12,721	45.6	1,417	19.7	3,085	19.3
Yes	15,197	54.4	5,785	80.3	12,906	80.7
Ever exposed to most highly correlated pesticides with metolachlor						
Cyanazine	7,447	26.7	3,626	51.5	9,454	60.4
EPTC	2,688	9.7	1,857	26.7	5,595	36.2
Alachlor	10,057	36.2	4,742	66.9	11,206	71.3
Imazethapyr	6,935	25.1	3,867	55.1	10,356	66.3
Trifluralin	9,222	33.9	4,721	67.0	11,824	75.5

The term "private applicators" refers primarily to farmers and "commercial" refers to professional pesticide applicators.

[1] 1st quartile of lifetime exposure-days (years of use × days per year).

[2] 2nd, 3rd, 4th quartiles of lifetime exposure-days (years of use × days per year).

The Poisson regression RRs of selected cancers with exposure to metolachlor are presented in Table II; the first column presents results for tertiles of lifetime days, and the second column presents results for tertiles of intensity-weighted lifetime days. Results displayed are for comparison to the low-metolachlor exposed applicators. For all cancers combined, all lymphohematopoetic cancers, prostate cancer, lung cancer and colon cancer we had enough case numbers (at least 5 in each category) to split the top tertile at the median and present results for that categorization. We found no association for all cancers combined. Prostate cancer was the most frequent cancer ($n = 299$). We detected a statistically significant decreased risk for prostate cancer in the upper half of the third tertile of lifetime days (RR = 0.59; 95%CI, 0.39-0.89) and in the second tertile of intensity-weighted lifetime days (RR = 0.66; 95%CI, 0.45-0.97); however, the test for trend was not significant for either of the two exposure metrics. For lung cancer, we found a nonsignificant, increased risk for applicators in the upper half of the third tertile of lifetime days (RR = 2.37; 95%CI, 0.97-5.82), and the test for trend was significant ($p = 0.03$). However, there was no association for intensity-weighted lifetime days. We found no evidence of increased risks for cancers of the oral cavity, colon, lymphohematopoetic system or for non-Hodgkin lymphoma. Using the non-metolachlor exposed applicators as the reference group, we detected null associations for all the cancer sites analyzed (data not shown). An analysis that was restricted to private applicators living in Iowa yielded results similar to those reported in Table II. The results for North Carolina were unstable as a result of smaller numbers of cases.

Table II. Rate Ratios[1] From Poisson Regressions For Selected Cancers[2] by Tertiles[3] of Lifetime Exposure-Days and Intensity-Weighted Lifetime Exposure-Days to Metolachlor[4] Among Agricultural Health Study Cohort Applicators with Low-Metolachlor Exposed Applicators as The Referent

	Lifetime days[6]				Intensity weighted lifetime days[7]			
Cancer site	*n*[5]	RR	95% CI	*p*-trend	*n*[5]	RR	95% CI	*p*-trend
All cancers								
T1	225	1.00			229	1.00		
T2	221	1.00	(0.83-1.21)		214	0.95	(0.78-1.15)	
$T3_L$	117	1.05	(0.83-1.32)		113	0.83	(0.65-1.07)	
$T3_U$	117	1.01	(0.78-1.30)	*0.98*	124	0.93	(0.72-1.21)	*0.72*
	680				680			
Oral cavity								
T1	6	1.00			9	1.00		
T2	9	1.51	(0.51-4.46)		6	0.63	(0.20-1.97)	
T3	6	1.39	(0.41-4.71)	*0.76*	6	0.83	(0.24-2.87)	*0.83*
	21				*21*			
Colon								
T1	18	1.00			21	1.00		
T2	15	0.89	(0.44-1.79)		14	0.73	(0.36-1.46)	
$T3_L$	13	1.58	(0.75-3.33)		8	0.71	(0.29-1.72)	
$T3_U$	11	1.30	(0.55-3.08)	*0.32*	14	1.24	(0.53-2.88)	*0.48*
	57				57			
Lung								
T1	13	1.00			12	1.00		
T2	11	1.02	(0.45-2.30)		16	1.44	(0.67-3.11)	
$T3_L$	10	1.89	(0.79-4.48)		8	1.38	(0.51-3.72)	
$T3_U$	12	2.37	(0.97-5.82)	*0.03*	10	1.65	(0.61-4.47)	*0.65*
	46				46			
Prostate								
T1	115	1.00			108	1.00		
T2	99	0.84	(0.63-1.10)		101	0.91	(0.69-1.21)	
$T3_L$	47	0.79	(0.55-1.13)		46	0.66	(0.45-0.97)	
$T3_U$	38	0.59	(0.39-0.89)	*0.21*	44	0.67	(0.44-1.01)	0.38
	299				299			
All lymphohematopoetic cancers								
T1	33	1.00			28	1.00		
T2	21	0.58	(0.33-1.03)		20	0.71	(0.38-1.32)	
$T3_L$	8	0.41	(0.18-0.91)		18	0.95	(0.47-1.89)	
$T3_U$	19	0.87	(0.49-1.67)	*0.50*	15	0.79	(0.37-1.69)	0.65
	81				81			
Non-Hodgkin lymphoma								
T1	14	1.00			13	1.00		

T2	11	0.76 (0.34-1.71)		10	0.69	(0.29-1.67)	
T3	11	0.68 (0.28-1.65)	*0.48*	13	1.00	(0.37-2.69)	0.70
	36			36			

[1] Adjusted for age, sex, race, smoking, alcohol, applicator status (private or commercial) family history of cancer, state of residence, and the most highly correlated pesticides with metolachlor.

[2] Cancers for which there were at least 20 exposed cases and 5 exposed cases in each category after accounting for missing covariate data.

[3] Top tertile split for all cancers combined, colon, lung, prostate, and all lymphohematopoetic cancers.

[4] Total number exposed to metolachlor: 22,781.

[5] Numbers of cancer-specific cases entered into the final models in each tertile of metolachlor exposure.

[6] Tertiles for lifetime days: 2.5-20, 21-56, >56; when top tertile split, T_{3L}: >56-116, T_{3U}: >116.

[7] Tertiles for intensity weighted lifetime days: 0.5-103, >103-362, >362; when top tertile split, T_{3L}: >362-924, T_{3U}: >924.

The results for other cancer sites are not presented in Table II, because there were fewer than 20 exposed cases or fewer than 5 cases in each exposure category. Rectum cancer was one of these sites, because although there were 25 exposed cases, there were only 4 cases in the lowest tertile of lifetime days exposure ($N_{2nd\ tertile}$ = 8, $N_{3rd\ tertile}$ = 13) and two cases in the lowest tertile of intensity-weighted lifetime days exposure ($N_{2nd\ tertile}$ = 8, $N_{3rd\ tertile}$ = 11). We found statistically significant elevated RRs for the highest tertile of lifetime days (RR = 4.04; 95%CI, 1.02-15.94; *p*-trend = 0.03). The RR results for intensity-weighted lifetime days were also elevated, though not statistically significant (3rd tertile: RR = 5.16; 95%CI, 0.97-27.42; *p*-trend = 0.31). When we split the upper tertile in half, the trends were similar. Using the non-metolachlor exposed applicators as the reference group, results for rectum cancer were generally null.

Discussion

We found no strong associations between any cancer sites and metolachlor exposure, whether the lifetime days or the intensity-weighted lifetime days metric was used in the analysis or whether the lowest metolachlor-exposed group or the nonexposed group was used as the referent. There were statistically significant estimates for prostate cancer which showed a decreased risk in the highest lifetime days exposure category and in the second tertile of intensity-weighted lifetime days exposure; however the test for trend was not significant, and the results for intensity-weighted lifetime days were weaker and mostly null, as were the results for the analysis using nonexposed applicators as the referent. To investigate these findings further, we stratified by education level (high school diploma or GED; at least some college), which we used as an indicator of socio-economic status. We found that the RRs were similar for applicators in both groups.

There was a marginally significant estimate in the highest lifetime days exposure category for lung cancer, and the test for trend was significant. To account more fully for smoking, we stratified the population into never smokers, former smokers and current smokers. Among never smokers the RR for lung cancer in the highest category of lifetime days was lower than that for former or current smokers, but was still elevated. However, the number of exposed, nonsmoker cases was small (*n* = 3) and the confidence interval wide: (RR = 1.65; 95% CI, 0.09-29.25) for nonsmokers, (2.61; 95%CI, 0.78-8.69) for former smokers, and (RR = 2.10; 95%CI, 0.67-6.62) for current smokers. Clearly, although we controlled for smoking in our models, it is a strong risk factor for lung cancer, and residual confounding from smoking is still possible. We also explored the impact of different approaches to modeling smoking, but results were not different from those presented. Alavanja *et al.* also investigated smoking in greater detail and showed that residual confounding by smoking was unlikely to explain elevated lung cancer RRs. Although there was no evidence of increased lung cancer risk with increasing intensity-weighted lifetime days of exposure, the intensity algorithm, which weighs dermal exposure more heavily than inhalation exposure, may not be an improvement over the lifetime days measure and may have introduced measurement error.

The human data for metolachlor is sparse. An association between lung cancer and metolachlor use in this cohort was reported by Alavanja *et al.*[3] based on fewer years of follow-up. In that study, using a nested case-

control design and logistic regression, an increased risk for lung cancer was found with metolachlor lifetime days for 3 higher exposure quartiles when compared with a low-exposed group: OR = 1.0, 1.6, 1.2, 5.0; *p*-trend = 0.0002 and a less consistent trend based on the nonexposed as the referent: OR = 1.0, 0.6, 1.0, 0.9, 4.1; *p*-trend = 0.015. Those findings do not differ meaningfully from the findings we present. When we re-ran our analyses as logistic regressions, using the same cut-points of exposure and the same covariates, we got very similar results to those of Alavanja *et al.*[3]; slight differences are expected since our study had one year additional follow-up.

A study by Lee *et al.*[5] found a significant increasing trend for incidence of all lymphohematopoetic cancers associated with lifetime days exposure and intensity-weighted lifetime days exposure to another chloroacetanilide herbicide, alachlor. We did not find any evidence of an association between metolachlor exposure and all lymphohematopoetic cancers or for NHL. The case numbers for leukemia, multiple myeloma and Hodgkin disease were too small to analyze. There is no previous human data on an association between metolachlor and other cancers. Our findings for rectum cancer are intriguing but are based on small numbers and must be interpreted with caution.

The carcinogenicity of metolachlor is thought to involve a complex metabolic activation pathway leading to a DNA-reactive dialkylbenzoquinone imine; however, the toxicological activity of metolachlor in humans is unclear. Metolachlor has been evaluated for carcinogenic activity in both rats and mice and for mutagenic activity *in vitro*. No treatment-related carcinogenic effects were observed in two chronic studies in Charles River CD-1 mice;[22][23][24][25] however a significant increase in liver neoplastic nodules was observed in female BR albino rats at the highest dose level (equivalent to 150 mg/kg/day).[22][23][24][25]

Classified by the EPA as a Group C "possible human carcinogen," metolachlor is not listed in the 11th report on carcinogens of the National Toxicology Program,[26] and so far its carcinogenicity has not been evaluated by the International Agency for Research on Cancer. Not all formulations of metolachlor have been found to be equal in toxicity. A study of 2 different metolachlor products, Dual™ and VUCHT 524™, found that the latter strongly induced growth of Syrian hamster embryo cells, while the former was inactive.[14] We cannot distinguish between metolachlor products used by farmers in our cohort, because individuals were asked if they had used "Dual, Cycle or other metolachlor products." If the toxicity of the products vary, then our results may underestimate the effects of a more toxic compound and overestimate the associations of less toxic compounds. In 1999 the major manufacturer of metolachlor replaced it with a reduced-risk herbicide, S-metolachlor; our data reflect metolachlor only, since our data for this analysis were collected prior to 1999.[27]

The AHS has several important strengths. It is the largest study of pesticide applicators exposed to metolachlor to date. Exposure information was gathered prior to cancer diagnosis, thereby minimizing recall bias. In general, farmers provide reliable information and considerable detail regarding their pesticide application history.[19][28][29][30] The AHS cohort consists of licensed pesticide applicators who are responsible for a thorough understanding of pesticide regulations and for the purchase and application of chemicals.[31] Recall of pesticide use by the AHS cohort has been shown to be consistent with the dates these pesticides came onto the market.[31] Comprehensive questionnaire data was used to quantify metolachlor exposure levels, providing discrimination between high and low exposures, rather than defining exposure as 'ever used' metolachlor. In addition, detailed information on the use of many common pesticides and lifestyle characteristics allowed us to adjust for potential confounding factors.

Certain limitations of our data hinder the inferences we can make regarding metolachlor and its association with specific cancers. Although the AHS cohort is large and there were 23,193 participants reporting metolachlor use, the small numbers of certain cancers occurring during the 7.33 year average follow-up period impacted precise effect estimation. Even for the more common tumors, case numbers limited the number of subpopulation categories that could be analyzed. In addition, most metolachlor applicators were male (99%), precluding our ability to assess the association between metolachlor exposure and female cancers. Our analysis provides limited information on the timing of pesticide use in relation to disease. Additionally, with only 7.33 years of follow-up we are limited in our conclusions concerning latency and temporal changes in personal protective equipment. We will be able to better address these issues with increased follow-up and exposure data from subsequent phases of the AHS. Although our study used more detailed exposure estimates than did earlier studies, a source of variation is that the hours an applicator worked in a day of pesticide application could vary considerably. Later phases of the AHS will enable us to reduce this source of variation.

Despite the limitations noted earlier, our prospective study of cancer incidence among metolachlor-exposed pesticide applicators provided an opportunity afforded in few other studies to evaluate cancer risks associated with exposure to metolachlor, while adjusting for other common pesticide exposures and lifestyle factors. We

did not detect strong evidence for an association between metolachlor exposure and any of the cancer sites investigated. We intend to follow up on these results in the future, focusing specifically on prostate cancer and the histologic variety of lung cancer as more cases develop in the cohort.

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REFERENCES

1 EPA. Reregistration Eligibility Decision (RED) Metolachlor, U.S. Environmental Protection Agency, Office of Prevention, Pesticides, and Toxic Substances; Washington, D.C., 1995.

2 Coleman S, Linderman R, Hodgson E, Rose RL. Comparative metabolism of chloroacetamide herbicides and selected metabolites in human and rat liver microsomes. *Environ Health Perspect* 2000; **108**: 1151-7. Links

3 Alavanja MC, Dosemeci M, Samanic C, Lubin J, Lynch CF, Knott C, Barker J, Hoppin JA, Sandler DP, Coble J, Thomas K, Blair A. Pesticides and lung cancer risk in the agricultural health study cohort. *Am J Epidemiol* 2004; **160**: 876-85. Links

4 Alavanja MC, Samanic C, Dosemeci M, Lubin J, Tarone R, Lynch CF, Knott C, Thomas K, Hoppin JA, Barker J, Coble J, Sandler DP, et al. Use of agricultural pesticides and prostate cancer risk in the Agricultural Health Study cohort. *Am J Epidemiol* 2003; **157**: 800-14. Links

5 Lee WJ, Hoppin JA, Blair A, Lubin JH, Dosemeci M, Sandler DP, Alavanja MC. Cancer incidence among pesticide applicators exposed to alachlor in the Agricultural Health Study. *Am J Epidemiol* 2004; **159**: 373-80. Links

6 Barbash JE, Thelin GP, Kolpin DW, Gilliom RJ. Major Herbicides in Ground Water: results from the National Water-Quality Assessment. *J Environ Qual* 2001; **30**: 831-45. Links

7 Beilstein P. L5178Y/tk+/-mouse lymphoma mutagenicity test: CGA 24′705 techn. Final report. Report No. 831500, Unpublished study by Ciba-Geigy, MRID No. 00158929 reviewed in HED Doc. 006444 1984.

8 Cifone M. Evaluation of metolachlor technical in the in vivo/in vitro rat hepatocyte unscheduled DNA synthesis assay. Final report. Lab Project No. 8147: 10992-001, Unpublished study by Hazleton Biotechnologies, submitted to OPP by Ciba-Geigy, MRID No. 42043301 reviewed in HED Doc. 008880 1988.

9 Dierickx PJ. Glutathione-dependent cytotoxicity of the chloroacetanilide herbicides alachlor, metolachlor, and propachlor in rat and human hepatoma-derived cultured cells. *Cell Biol Toxicol* 1999; **15**: 325-32. Links

10 Hertner T. CGA-77102 technical: in vivo/invitro unscheduled DNA synthesis in rat hepatocytes: Lab Project No. 941062: 94-50: 300202, Unpublished study by Ciba-Geigy, MRID No. 43928928 reviewed in HED Doc. 012310 1995.

11 Hertner T. CGA-77102 technical: micronucleus test, mouse (OECD conform): Lab Project No. 941061, Unpublished study by Ciba-Geigy, MRID No. 4398926 reviewed in HED Doc. 012310 1995.

12 Osano O, Admiraal W, Klamer HJ, Pastor D, Bleeker EA. Comparative toxic and genotoxic effects of chloroacetanilides, formamidines and their degradation products on Vibrio fischeri and Chironomus riparius. *Environ Pollut* 2002; **119**: 195-202. Links

13 Roloff B, Belluck D, Meisner L. Cytogenetic effects of cyanazine and metolachlor on human lymphocytes exposed in vitro. *Mutat Res* 1992; **281**: 295-8. Links

14 Slamenova D, Dusinska M, Gabelova A, Bohusova T, Oravec C. An evaluation of three pesticides: piritione, supercypermethrin and metolachlor in transformation bioassays of BHK21 and hamster embryo cells. *Cell Biol Toxicol* 1992; **8**: 217-31. Links

15 Strasse F. Nucleus anomaly test in somatic interphase nuclei of Chinese hamster. Final report. Report No. 831498, Unpublished study by Ciba-Geigy, MRID No. 00142826 and 00158925 reviewed in HED Doc. 004725 and 006444, 1984.

16 Blair A, Zahm SH, Pearce NE, Heineman EF, Fraumeni JF. Clues to cancer etiology from studies of farmers. *Scandinavian J Work Environ Health* 1992; **18**: 209-15. Links

17 Alavanja MC, Sandler DP, McMaster SB, Zahm SH, McDonnell CJ, Lynch CF, Pennybacker M, Rothman N, Dosemeci M, Bond AE, Blair A. The Agricultural Health Study. *Environ Health Perspect* 1996; **104**: 362-9. Links

18 Alavanja MC, Sandler DP, McDonnell CJ, Lynch CF, Pennybacker M, Zahm SH, Mage DT, Steen WC, Wintersteen W, Blair A. Characteristics of pesticide use in a pesticide applicator cohort: the Agricultural Health Study. *Environ Res* 1999; **80**(Pt 1): 72-9. Links

19 Blair A, Tarone R, Sandler D, Lynch CF, Rowland A, Wintersteen W, Steen WC, Samanic C, Dosemeci M, Alavanja MC. Reliability of reporting on life-style and agricultural factors by a sample of participants in the Agricultural Health Study from Iowa. *Epidemiology* 2002; **13**: 94-9. Links

20 Dosemeci M, Alavanja MC, Rowland AS, Mage D, Zahm SH, Rothman N, Lubin JH, Hoppin JA, Sandler DP, Blair A. A quantitative approach for estimating exposure to pesticides in the Agricultural Health Study. *Ann Occup Hyg* 2002; **46**: 245-60. Links

21 StataCorp. *Stata reference manual: release 8*. College Station, TX: Stata Press, 2003.

22 Office of Pesticide Programs, Peer review of metolachlor, HED Doc 007809. US Environmental Protection Agency, 1985.

23 Office of Pesticide Programs, Carcinogenicity peer review of metolachlor (3rd), HED Doc 009074. US Environmental Protection Agency, 1991.

24 Office of Pesticide Programs, Second peer review of metolachlor, HED Doc 010490. US Environmental Protection Agency, 1993.

25 Office of Pesticide Programs, Carcinogenicity peer review of metolachlor (4th), HED Doc 007809. US Environmental Protection Agency, 1994.

26 National Institute of Environmental Health Science. Report on Carcinogens, Eleventh Edition. US Department of Health and Human Services, Public Health Service, National Toxicology Program (http://ntp.niehs.nih.gov/ntp/roc/toc11.html), 2005.

27 Pointius NL. *Metolachlor, Regulatory Briefing. Rural Water Partnership Fund Regulatory Briefing* (http://www.nrwa.org/whitepapers/reg/regmt.doc). Pontius Water Consultants, Inc., Lakewood, CO, 2002.

28 Blair A, Stewart PA, Korss B, Ogilvie L, Burmeister LF, Ward MH, Zahm SH. Comparison of Two Techniques to Obtain Information on Pesticide Use from Iowa Farmers by Interview. *J Agric Saf Health* 1997; **3**: 229-36. Links

29 Blair A, Zahm SH. Patterns of pesticide use among farmers: implications for epidemiologic research. *Epidemiology* 1993; **4**: 55-62. Links

30 Engel LS, Rothman N, Knott C, Lynch CF, Logsden-Sackett N, Tarone RE, Alavanja MC. Factors associated with refusal to provide a buccal cell sample in the Agricultural Health Study. *Cancer Epidemiol Biomarkers Prev* 2002; **11**: 493-6. Links

31 Hoppin JA, Umbach DM, London SJ, Alavanja MC, Sandler DP. Chemical predictors of wheeze among farmer pesticide applicators in the Agricultural Health Study. *Am J Respir Crit Care Med* 2002; **165**: 683-9. Links